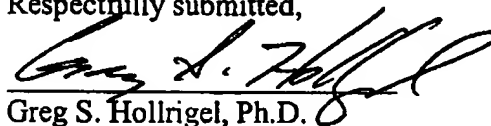


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If a telephone interview would be of assistance in advancing prosecution of the subject application, Applicant's undersigned representative invites the Examiner to telephone either Quan Nguyen or Greg S. Hollrigel, Ph.D., at the number provided below.

Date: 8/13/01

Respectfully submitted,



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VERSION WITH MARKINGS SHOWING CHANGES BEING MADE

In the Specification:

The paragraph beginning at page 1, line 10 has been amended as follows:

--This application is a division of United States Application Serial No. 08/196,082, filed March 3, 1994, now abandoned, which is a continuation of International Application No. PCT/US92/07381, filed August 28, 1992; and a continuation-in-part of United States Patent Application Serial No. 08/182,117, filed January 27, 1994, now abandoned, which is a continuation-in-part of International Application No. PCT/US92/06283, filed July 30, 1992, United States Patent Application Serial No. 07/750,579, filed August 28, 1991, now abandoned, and United States Patent Application Serial No. 07/738,040, filed July 30, 1991; now abandoned, which is a continuation-in part of United States Patent Application Serial No. 07/559,955, filed July 31, 1990, now abandoned, which is a continuation-in-part of United States Patent Application Serial No. 07/472,070, filed January 30, 1990, now abandoned, which is a continuation-in-part of United States Patent Application Serial No. 07/388,044, filed July 31, 1989, now abandoned.--

The paragraph beginning at page 12, line 17 has been amended as follows:

--Figure 6. Nucleotide sequence of human TPO gene after site-directed mutagenesis (SEQ ID NO: 1). The mutations incorporated two stop codons, as well as an EcoR1 site for confirmation, in the region immediately upstream from the transmembrane region of the human TPO gene.--

The paragraph beginning at page 12, line 22 has been amended as follows:

--Figure 7. cDNA sequence (SEQ ID NO: 2) and derived amino acid sequence (SEQ ID NO: 3) of human thyroid peroxidase (Magnusson, R.P., et al., Mol. Endocrinol. 1:856-861 (1987)). FIG. 7A depicts the cDNA sequence of human thyroid peroxidase from nucleotides 1 to 486, and the amino acid sequence of human thyroid peroxidase from amino acids 1 to 134. FIG. 7B depicts the cDNA

sequence of human thyroid peroxidase from nucleotides 487 to 972, and the amino acid sequence of human thyroid peroxidase from amino acids 135 to 296. FIG. 7C depicts the cDNA sequence of human thyroid peroxidase from nucleotides 973 to 1458, and the amino acid sequence of human thyroid peroxidase from amino acids 297 to 458. FIG. 7D depicts the cDNA sequence of human thyroid peroxidase from nucleotides 1459 to 1945, and the amino acid sequence of human thyroid peroxidase from amino acids 459 to 620. FIG. 7E depicts the cDNA sequence of human thyroid peroxidase from nucleotides 1946 to 2484, and the amino acid sequence of human thyroid peroxidase from amino acids 621 to 800. FIG. 7F depicts the cDNA sequence of human thyroid peroxidase from nucleotides 2485 to 3072, and the amino acid sequence of human thyroid peroxidase from amino acids 801 to 933. Asterisks (*) indicate potential glycosylation sites. The carets (^) at nucleotides 2884, 2885, and 2886 indicate an in phase termination codon. The carets (^ ^) at nucleotides 3042 to 3048 indicate a polyadenylation signal near the 3'-end.--

The paragraph beginning at page 15, line 14 has been amended as follows:

--Figure 18. Determination of the epitope for the anti-microsomal/TPO monoclonal antibody 20.10. The nucleotide sequences of the 5' - and 3'-ends were determined for 14 clones selected from the hTPO cDNA fragment library. These boundaries are annotated by the numbers assigned to the nucleotides in hTPO previously reported (Magnusson, R.P., et al., Mol. Endocrinol. 1:856-861 (1987)). The smallest region of overlap between all 14 clones is from 881-927 b.p. The first two nucleotides in this span do not constitute a complete codon, so the epitope area can be defined as between 883-927 b.p. (SEQ ID NO: 4), corresponding to the derived amino acid sequence shown (SEQ ID NO: 5).--

The paragraph beginning at page 15, line 26 has been amended as follows:

--Figure 19. Determination of the epitope recognized by TPO Mab 47. The nucleotide sequences of the 5'- and 3'-prime ends were determined for 18 clones in the TPO cDNA fragment library (see Material and Methods) recognized by Mab 47. The smallest region of overlap between all 18

clones is from 2219-2247 (SEQ ID NO: 6) basepairs in the human TPO cDNA sequence, coding for the indicated amino acids (SEQ ID NO: 7).--

The paragraph beginning at page 16, line 13 has been amended as follows:

--Figure 21: Binding affinity of Fab fragment SP2 for recombinant human thyroid peroxidase (TPO). Brackets indicate the mean \pm the range of duplicate densitometric values obtained for each TPO concentration in a representative experiment. Comparable results were obtained in two additional experiments.

[Figures 27A to 27B] Figures 22A to 22B: Fig.22A. Effect of increasing molar (M) concentrations of TPO, lactoperoxidase (LPO) or myeloperoxidase (MPO) on the binding of 125 I-TPO by SP1.2. Background binding in the absence of Fab fragments (2%) was subtracted. Fig.22B. Competition inhibition by unlabeled TPO of radiolabeled TPO binding to the Fab fragments. In the absence of unlabeled TPO, binding values for the three Fab fragments were 13-15%. Background of 2% was subtracted. Dissociation constants (K_d) were determined by Scatchard analysis (Scatchard, G., "The attractions of proteins for small molecules and ions," Ann. NY Acad. Sci. VOL 51:660-672 (1949)) and are:

$$SP2 = 8.3 \times 10^{-11} M; SP4 = 2.2 \times 10^{-10} M; SP5 = 3 \times 10^{-11} M$$

[Figure 28] Figure 23: Inhibition by increasing molar (M) concentrations of SP1.2 on the binding to 125 I-TPO by serum TPO autoantibodies. The mean values (\pm S.E.M.) obtained for sera from 11 patients are shown by solid circles. Background binding by serum from a TPO autoantibody negative donor was not subtracted and is shown by the open circles (mean \pm S.E.M. of 3 experiments).

[Figures 29A to 29C] Figures 24A to 24C: Competition ELISA for binding to TPO between the SP1.2 Fab fragment and TPO autoantibodies of different IgG subclasses. Figs. 24A to 24C show data obtained with three different patients. TPO autoantibody levels are shown as the O.D. readings

measured at 492nm. Background O.D. values obtained for TPO autoantibody-negative serum were <0.05 . SP1.2 (M); molar concentration of SP1.2.

[Figures 30A to 30B] Figures 25A to 25B: Effect of denaturation of TPO on SP Fab fragment binding. Binding of SP1.2, SP1.4 and SP1.5 Fig. 25A or mouse monoclonal antibody #40.28 Fig. 25A was measured to native or denatured TPO by ELISA. Binding is shown as the O.D. value at 492nm. Background O.D. values for TPO autoantibody negative serum and control murine ascites were <0.05 .

[Figure 33] Figure 26: Binding domains on TPO for the SP1.2, SP4.6, SP1.20 F(ab)s. 125 I-TPO was preincubated in the absence or presence of increasing concentrations of SP4.6, SP1.20 or SP1.2 [Free F(ab)]. The ability of these complexes to bind to immobilized SP1.2 was then determined. The results are expressed as % 125 I-TPO bound after subtraction of background values ($\sim 2\%$) obtained using buffer alone.

[Figures 36A to 36D] Figures 27A to 27D: Domains on TPO recognized by F(ab)s. Increasing concentrations of one F(ab) were pre-incubated with radiolabeled TPO and then added to a second, immobilized F(ab) (Methods). The immobilized F(ab) was TR1.9 Fig. 27A, TR1.7 Fig 27C and SP1.5 Fig 27D. The ability of the free F(ab) to inhibit binding to itself is shown by the open circles. Confirmation of the binding potency of the free F(ab)s was determined concurrently in each experiment. A representative control Fig. 27B for the experiment in Fig. 27A. is shown.

[Figure 37] Figure 28: Schematic representation of the binding domains on TPO for the expressed F(ab)s.

[Figure 38A to 38C] Figures 29A to 29C: Domains on TPO recognized by autoantibodies in 3 representative sera Figs. 29A ,29B and 29C from patients with autoimmune thyroid disease. F(ab)s WR1.7 and TR1.9, alone or in combination, were used to compete for serum autoantibody binding to radiolabeled TPO (Methods).--

The paragraph beginning at page 48, line 21 has been amended as follows:

--The non-coding strand of human TPO cDNA, in the phagemid Bluescript (Stratagene, San Diego, CA), was used as a template for oligonucleotide-directed mutagenesis. A 52 bp mutagenic primer (5' -AGGCTCCCTCGGGTGAATTCCCATGTAGCTGGCTGCTCTGCTGATCG-3'), (SEQ ID NO: 8) synthesized by the molecular Genetics Core Facility, San Francisco Veterans' Administration Medical Center, was designed to generate two stop codons directly upstream of the putative membrane-spanning region of the protein. Thus, TGA and TAG codons were created at 2629-2631 bp and 2641-2643 bp in human TPO cDNA (Magnusson, R.P., et al., Mol. Endocrinol. 1:856-861 (1987)), respectively. The cDNA sequence of human TPO as published on page 857 as Fig. 2 in Magnusson, R.P., et al., Mol. Endocrinol. 1:856-861 (1987) is as follows:

gaggcaattgagggcgcccatttcagaagagttacagccgtgaaaattactcagcagtgca 60
gttggctgagaagaggaaaaagaatgagagcgctggctgtgctgtctgtcacgctggtt 120
atggcctgcacagaagccttcttcccccttcattctcgagagggaaagaactcctttgggga 180
aagcctgaggagtctcgtgtctctagcgtcttggaggaaagcaagcgccctggtggacacc 240
gcatgtacgccacgatgcagagaaacctcaagaaaagaggaatcctttctggagctcag 300
cttctgtctttttccaaacttctgagccaacaagcggagtgtattgcccgagcagcagag 360
ataatggaaacatcaatacaagcgatgaaaagaaaagtcaacctgaaaactcaacaatca 420
cagcatccaacggatgctttatcagaagatctgctgagcatcattgcaaacatgtctgga 480
tgtctcccttacatgctgcccccaaatgccccaaacacttgccctggcggaacaaatacagg 540
cccatcacaggagcttgcaacaacagagaccacccagatggggcgccctccaacacggcc 600
ctggcacgatggctccctccagtctatgaggacggcttcagtcagccccgaggctggaac 660
cccggcttcttgtaaacgggttcccactgcccccggtccgggaggtgacaagacatgtc 720
attcaagtttcaaatgaggttggtcacagatgatgaccgctattctgacctcctgatggca 780
tggggacaatacatcgaccacgacatcgcggttcacaccacagagcaccagcaaagctgcc 840
ttcgggggaggggtctgactgccagatgacttgtgagaacaaaaacccatgttttcccata 900
caactcccggaggaggcccggccggccgggacccgctgtctgcccttctaccgctct 960

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tccggccgcctgcggcaccggggaccaaggcgcgctctttgggaacctgtccacggccaac 1020
ccgaggcagcagatgaacgggttgacctcgttcctggacgcgtccaccgtgtatggcagc 1080
tccccggccctagagaggcagctgcggaactggaccagtgccgaagggtgctccgcgtc 1140
cacggccgcctccgggactccggccgcgcctacctgcccttcgtgccgccacgcgcgcct 1200
gcgccctgtgcgcccagagcccggcaacccccggagagacccgcgggccctgcttcctggcc 1260
ggagacggccgcgccagcgaggtcccctccctgacggcaactgcacacgctgtggctgcgc 1320
gagcacaaccgcctggccgcggcgctcaaggccctcaatgcgcactggagcgcgagcgc 1380
gtgtaccaggaggcgcgcaaggctcgtgggcgctctgcaccagatcatcaccctgagggat 1440
tacatccccaggatcctgggacccgaggccttcagcagtacgtgggtccctatgaaggc 1500
tatgactccaccgccaacccccactgtgtccaacgtgttctccacagccgccttcgccttc 1560
ggccatgccacgatccaccgcgtggtgaggaggctggacgccagcttcaggagcacccc 1620
gacctgcccgggctgtggctgcaccaggctttcttcagcccatggacattactccgtgga 1680
ggtggtttggaccactaatacagaggccttcttgcaagaccagccaaactgcagggtgcag 1740
gatcagctgatgaacgaggagctgacggaaaggctctttgtgctgtccaattccagcacc 1800
ttggatctggcgtccatcaacctgcagaggggcccgggaccacgggctgccaggttacaat 1860
gagtggaggaggattctgcggcctgcctcgctggagacccccgcgtgacctgagcacagcc 1920
atcgccagcaggagcgtggccgacaagatcctggacttgataagcatcctgacaacatc 1980
gatgtctggctgggaggcttagctgaaaacttcctccccagggtcggacagggccctg 2040
tttgctgtctcattgggaagcagatgaaggctctgcgggacgggtgactggttttggtgg 2100
gagaacagccacgtcttcacggatgcacagaggcgtgagctggagaagcactccctgtct 2160
cgggtcatctgtgacaacactggcctcaccagggtgcccatggatgccttccaagtcggc 2220
aaattccccgaagactttgagtcttggtgacagcatcactggcatgaacctggaggcctgg 2280
agggaaacctttcctcaagacgacaagtgtggcttcccagagagcgtggagaatggggac 2340
tttgtgactgtgaggagtctgggaggcgctgctggtgtattcctgccggcacgggtat 2400
gagctccaaggccgggagcagctcacttgacccaggaaggatgggatttccagcctccc 2460
ctctgcaaagatgtgaacgagtgtgcagacggtgccacccccctgccacgcctctgcg 2520
aggtgcagaaacaccaaaggcggttccagtgtctctgcgcggacccctacgagttagga 2580
gacgatgggagaacctgcgtagactccgggaggctccctcgggtgacttgatctccatg 2640
tcgctggctgctctgctgatcggaggcttcgcaggcttcacctgcaggtgatttgagg 2700

tggacacgcactggcactaaatccacactgcccattctcggagacaggcggaggaactccc 2760
gagctgagatgcggaagcaccaggccgtagggacctcaccgcagcgggcccgcagctcag 2820
gactcggagcaggagagtgtctgggatggaaggccgggatactcacaggctgccgagagcc 2880
ctctgagggc aaagtggcaggacactgcagaacagcttcatgttcccaaatcaccgtac 2940
gactcttttccaaacacaggcaaattcggaatcagcaggacgactgttttcccaacacgg 3000
gtaaatctagtaccatgtcgtagttactctcaggcatggatgaataaatgttatagctgc 3060
aaaaaaaaaaaa 3072 (SEQ ID NO: 2).

For convenient screening of mutants, an Eco RI restriction site (GAATTC, at 2630-2635 bp) was created together with the first (TGA) stop codon. The mutagenesis procedure was performed according to the protocol of the manufacturer (Muta-gene phagemid in vitro mutagenesis kit, Biorad, Richmond, CA) to generate the plasmid pHTPO(M1)-BS.--

The paragraph beginning at page 69, line 2 has been amended as follows:

--TPO cDNA fragment library construction: A full-length (3.05 kb) cDNA clone as described above for human thyroid peroxidase was released from its Bluescript vector (Stratagene, San Diego, CA.) by digestion with EcoRI (BRL Laboratories, Gaithersburg, MD) and NotI (Boehringer, Mannheim, West Germany). Because both vector and insert are of similar length, the Bluescript was further digested with ScaI (New England Biolabs, Beverly, MA.). The TPO cDNA was purified by agarose gel electrophoresis and electroelution. The cDNA was then digested (6 minutes at room temperature) into small random-sized fragments with DNAase I (0.1 ng DNase/ug cDNA) (BRL) in 20 mM Tris-HCl, pH 7.5, 1.5 mM MnCl₂ and bovine serum albumin, 100 ug/ml. After electrophoresis in 2% SeaPlaque agarose (FMC Bio Products, Rockland, ME), TPO cDNA fragments 200-500 b.p. in length were recovered by electroelution. The ends of the fragments were blunted with the Klenow fragment of DNA polymerase I, and ligated to EcoRI linkers (GAATTCGGCACGAG) (SEQ ID NO: 9) containing a nonphosphorylated EcoRI cohesive end and a phosphorylated blunt end (Pharmacia, Piscataway, NJ). After phosphorylation with polynucleotide kinase, excess linkers were removed by electrophoresis in 2% SeaPlaque agarose.

The linker-ligated cDNA was again size-selected (200-500 b.p.), electroeluted, ethanol precipitated and ligated into EcoRI-cut lambda-Zap vector (Stratagene). After packaging (Giga-Pak Gold, Stratagene), the library was amplified in XL1-blue cells (Stratagene). cDNA insert sizes were confirmed by the polymerase chain reaction (PCR) (Saiki, R.K., et al., Science 239:487-491 (1988)) using the Bluescript reverse and -20 primers. PCR analysis of the "C2" hTPO cDNA region (Libert, F., et al., EMBO J. 6:4193-4196 (1987); Ludgate, M., et al., J. Clin. Endocrinol. Metab. 68:1091-1096 (1989)) in the TPO cDNA fragment library was performed using two oligonucleotide 22-mer primers (5'- GGTTACAATGAGTGGAGGGAGT (SEQ ID NO: 10) and 5' - GTGGCTGTTCTCCCACCAAAAC) (SEQ ID NO: 11) spanning the region 1852-2112 b.p. in hTPO (17). PCR (30 cycles) was for 1 minute at 94°C, 2 minutes at 55°C and 1 minute at 72°C. For screening the library, the PCR-generated DNA was labeled with ^{32}P - α CTP to a specific radioactivity of 0.8×10^9 cpm/ug DNA using the random primer method (Multiprime; Amersham, Arlington Heights, IL). The screening procedure employed standard techniques (Maniatis, T., et al., Molecular Biology: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982)), with final washes of 30 minutes (x 2) at 55°C in 0.1 x SSC, 1% SDS buffer (1 x SSC in 150 NaCl, 15 mM Na citrate, pH 7.5). Autoradiography of the nitrocellulose filters was performed with Kodak XAR-5 film.--

The paragraph beginning at page 73, line 21 has been amended as follows:

--Screening of this library with the anti-microsomal antigen monoclonal antibody yielded 6-12 positive plaques per 1,000 plaques screened. Fourteen positive clones were randomly chosen for partial nucleotide sequencing to delineate the position of their TPO cDNA inserts relative to the entire TPO gene. Twelve of the 14 clones had cDNA inserts of 160-350 b.p. Two clones (U and Y) that had cDNA inserts slightly larger than the expected 500 b.p. maximum were found, upon nucleotide sequencing, to have double cDNA inserts. As an indication of the success of the procedure, all 14 clones recognized by the monoclonal antibody spanned the same region (746-1,150 b.p.) of the hTPO gene (Magnusson, R.P., et al., Mol. Endocrinol. 1:856-861 (1987)) (Figure 18). The maximum region common to all clones, and therefore an indication of a common

epitope, was between bases 881 and 927 (AA AAC CCA TGT TTT CCC ATA CAA CTC CCG GAG GAG GCC CGG CCG GCC) (SEQ ID NO: 12), corresponding to a derived amino acid sequence of only 15 residues (Asn Pro Cys Phe Pro Ile Gln Leu Pro Glu Glu Ala Arg Pro Ala) (SEQ ID NO: 5). Therefore, the epitope recognized by the monoclonal antibody lies within this 15 amino acid span.--

In the Claims:

The following claims have been added:

- 60. (new) A recombinant DNA sequence encoding a human thyroid peroxidase which is secreted from a cell, wherein the DNA has a stop codon at nucleotides 2629-2631 of SEQ ID NO: 2.--
- 61. (new) A recombinant DNA sequence consisting of nucleotides 1-2628 of SEQ ID NO: 2.--
- 62. (new) A recombinant DNA sequence consisting of nucleotides 85-2628 of SEQ ID NO: 2.--
- 63. (new) A recombinant DNA sequence encoding a human thyroid peroxidase that consists of amino acids 1 to 848 of the amino acid sequence shown in FIG. 7.--
- 64. (new) A plasmid vector designated ATCC accession number CRL 10250.--
- 65. (new) A DNA encoded by the vector of claim 64.--
- 66. (new) A vector which comprises the DNA sequence of claim 60.--
- 67. (new) A host cell transformed with the vector of claim 66.--

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Claims 38-59 have been canceled without prejudice.

14/53

2511

2538

GCA GAC GGT GCC CAC CCC CCC TGC CAC GCC ~~TAT~~ GCG AGG TGC AGA AAC ACC AAA
 Ala Asp Gly Ala His Pro Pro Cys His Ala Ser Ala Arg Cys Arg Asn Thr Lys

2565

2592

GGC GGC TTC CAG TGT CTC TGC GCG GAC CCC TAC GAG TTA GGA GAC GAT GGG AGA
 Gly Gly Phe Gln Cys Leu Cys Ala Asp Pro Tyr Glu Leu Gly Asp Asp Gly Arg

2619

2646

ACC TGC GTA GAC TCC GGG AGG CTC CCT CGG GTG ACT TGG ATC TCC ATG TCG CTG
 Thr Cys Val Asp Ser Gly Arg Leu Pro Arg Val Thr Trp Ile Ser Met Ser Leu

2673

2700

GCT GCT CTG CTG ATC GGA GGC TTC GCA GGT CTC ACC TCG ACG GTG ATT TGC AGG
 Ala Ala Leu Leu Ile Gly Gly Phe Ala Gly Leu Thr Ser Thr Val Ile Cys Arg

2727

2754

TGG ACA CGC ACT GGC ACT AAA TCC ACA CTG CCC ATC TCG GAG ACA GGC GGA GGA
 Trp Thr Arg Thr Gly Thr Lys Ser Thr Leu Pro Ile Ser Glu Thr Gly Gly Gly

2781

2808

ACT CCC GAG CTG AGA TGC GGA AAG CAC CAG GCC GTA GGG ACC TCA CCG CAG CGG
 Thr Pro Glu Leu Arg Cys Gly Lys His Gln Ala Val Gly Thr Ser Pro Gln Arg

2835

2862

GCC GCA GCT CAG GAC TCG GAG CAG GAG AGT GCT GGG ATG GAA GGC CGG GAT ACT
 Ala Ala Ala Gln Asp Ser Glu Gln Glu Ser Ala Gly Met Glu Gly Arg Asp Thr

2889

2916

CAC AGG CTG CCG AGA GCC CTC TGA GGG CAA AGT GGC AGG ACA CTG CAG AAC AGC
 His Arg Leu Pro Arg Ala Leu ^^^

2943

2970

TTC ATG TTC CCA AAA TCA CCG TAC GAC TCT TTT CCA AAC ACA GGC AAA TCG GAA

2997

3024

ATC AGC AGG ACG ACT GTT TTC CCA ACA CGG GTA AAT CTA GTA CCA TGT CGT AGT

3051

TAC TCT CAG GCA TGG ATG AAT AAA TGT TAT AGC TGC AAA AAA AAA AAA
 ^^^ ^^^

FIG.7F**SUBSTITUTE SHEET**